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Human Performance and Biosystems

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2013 AFOSR Spring Review
Portfolio Overview

NAME: Patrick O. Bradshaw, Ph.D.

BRIEF DESCRIPTION OF PORTFOLIO:

• **Human Performance and Biosystems** is a program that characterizes, models and explains the structural features, metabolic functions and gene regulatory mechanisms utilized by various biological systems to capture, transfer, convert, or store energy for the purpose of understanding and possibly improving the power output of the organism.

Sub-Areas: (1) BioSolar Hydrogen, (2) Biofuel Cells (Microbial and Enzymatic), (3) Photo-Electro-Magnetic Stimulation of Biological Responses (PEMB)

• **PEMB** is a program that characterizes, models and explains the stimulatory and inhibitory responses of biological systems to low-level exposures of photo-electro-magnetic stimuli. Potential long-term benefits may include accelerated recovery from mental fatigue and drowsiness, enhanced learning and training, cellular and noninvasive understanding of brain function.

Visionary Transformational AF Capabilities

Human Performance/Biosystems:
Photo-electro-magnetic Stimulation of Bio-Responses:

- **Electromagnetically Enhanced Cognition, Protection and Repair:**
  - low-level exposure with photo-electro-magnetic stimuli enhance cognitive functions, bio-molecular repair and bio-resiliency

Bioenergy:

- **Portable H_2 Fuel Generated from H_2O or Cellulose:**
  - Cheap, self-healing inorganic catalysts split water into H_2 and O_2
  - Engineered photosynthetic microbes produce H_2 fuel

- **Compact Power from Ambient Biomass:**
  - Efficient electron transport coupled with unique electrode architectures enhance power and energy densities of biofuel cells

Challenges, Opportunities and Breakthrough Examples

Natural Systems Research:

Challenge: Explain gene regulatory mechanisms of metabolic pathways

Payoffs: - enhanced energy density of microbial fuel cells (MFC)

Challenge: Understand mechanisms & kinetics of enzyme-catalyzed reactions

Payoffs: - enhanced energy density of enzymatic fuel cells (EFC)

Artificial Systems Research:

Challenge: Discover/fabricate, durable synthetic materials that mimic the enzymatic or structural functions in natural energy systems

Payoffs: - enhanced power and energy densities for EFC
- nanowire may be capable of transmitting electrical charge from natural system to artificial system

Challenge: Integrate and assemble nano-scale inorganic/organic/bio-materials

Payoffs: - ordered enzyme alignments for enhanced power densities in EFC
- enhanced electron transport and power density in biofuel cells

Overview of Topic Areas 3003

Human Performance/Biosystems

- Photo-Electro-Magnetic Stimulation of Biosystems
- Biomarkers, Physiological responses and toxicology
- Artificial Biology, explore non coding genetic information

Bioenergy: Alternative Energy

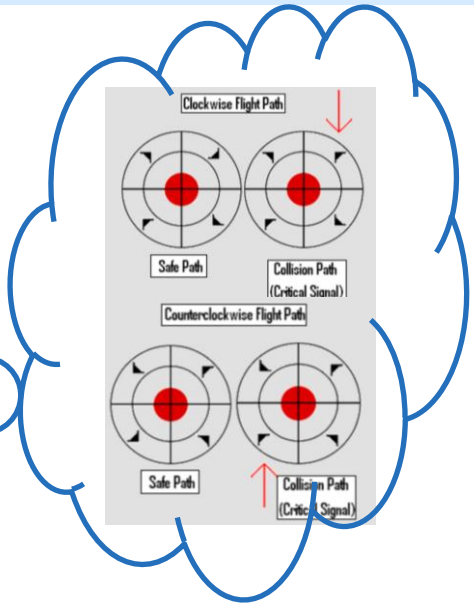
- Biofuels—Macro-scale Energy
  - Biosolar Hydrogen
  - Algal Oil for Jet Fuel
  - Artificial Biology
- Biofuel Cells—Micro-scale Energy
  - Enzymatic Fuel Cells
  - Microbial Fuel Cells
  - Artificial Photosynthesis

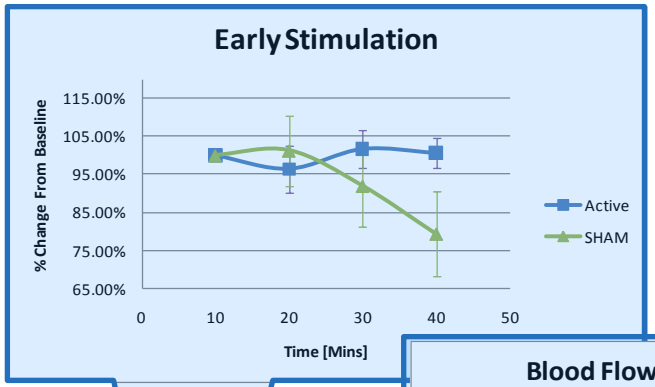
Electric Stimulation of the Brain, Hemodynamics and Sustained Attention:

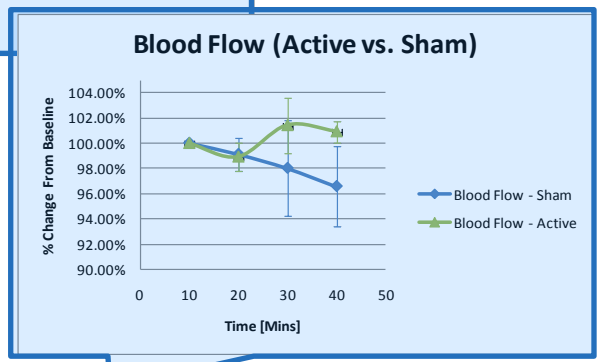
McKinley (AFRL/RH)

Objective: Quantify effects on human vigilance and hemodynamics due to non-invasive stimulation of the brain by low levels of direct current (1 mA).

NEW PROJECT 2011







Gordon et al., 2009

Astrocytes …?

rCBF

Moore & Cao, 2008

Anodal Stim. → ↑P(APs) → ↑rSO_2 → ↑Information processing → ↑Vigilance Perform. → ↑CO_2 → ↑rCBF

Potential Metrics

Merzagora et al., 2010

Helton et al., 2010

Hellige, 1993 & Warm et al., 2009

Transcranial Direct Current Stimulation (tDCS)

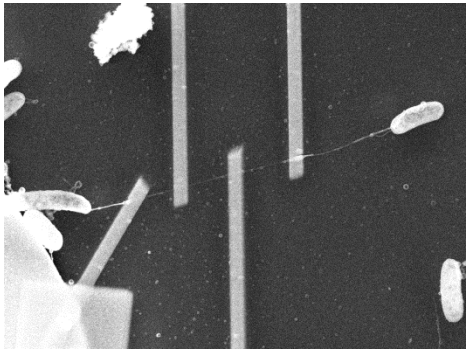
One lab task funded this year - Jankord

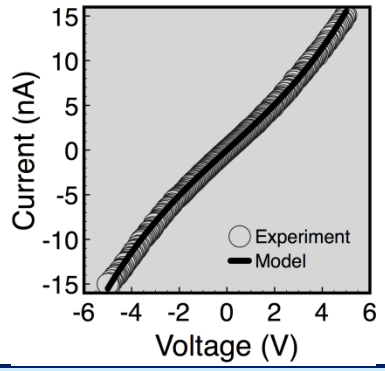
1 proposal funded – Bikson, CUNY
Thin brain slice pathway determination

1 Transcranial Magnetic Stimulation proposal for next year

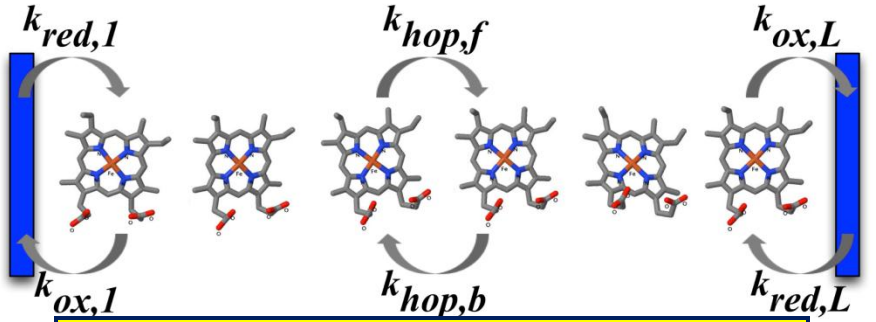
The Biophysical Mechanism of Extracellular Charge Transport: El-Naggar (USC)

(1) Electronic transport in bacterial nanowires was demonstrated using nanofabrication enabled approaches

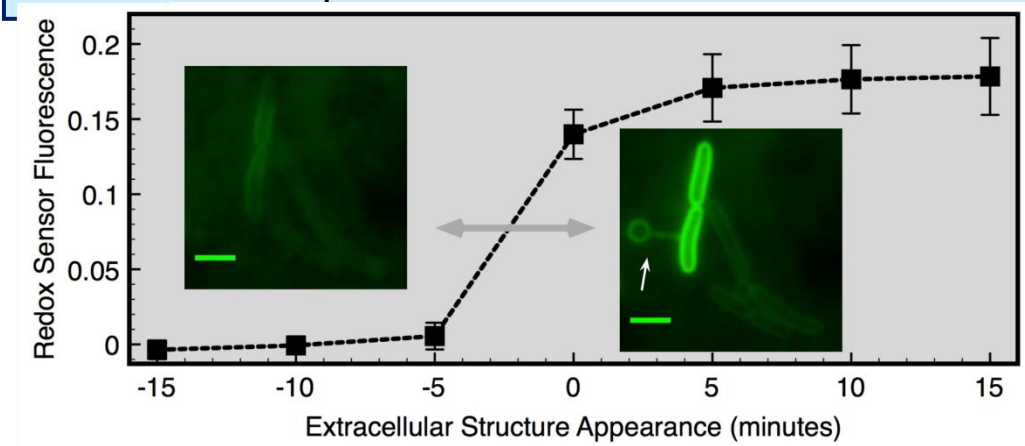




(2) Identified the biophysical mechanism responsible for long-distance extracellular charge transport: Multi-step hopping in microbial redox chains



(3) First ***in vivo*** demonstration of bacterial nanowires and outer-membrane vesicles enhancing the electron transfer and respiration of individual cells

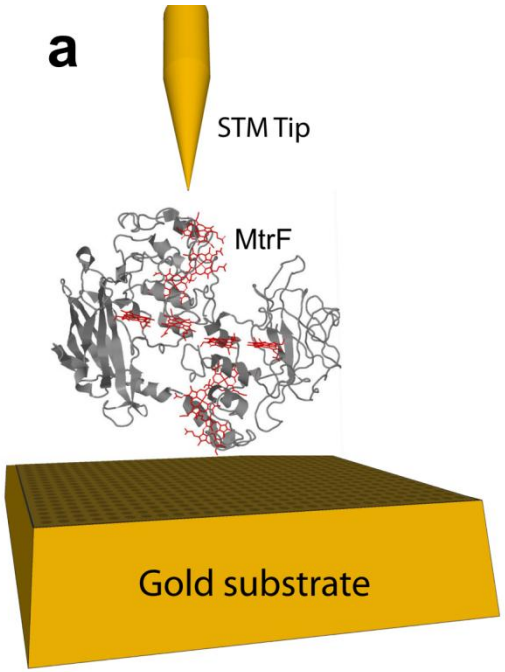


Outlook

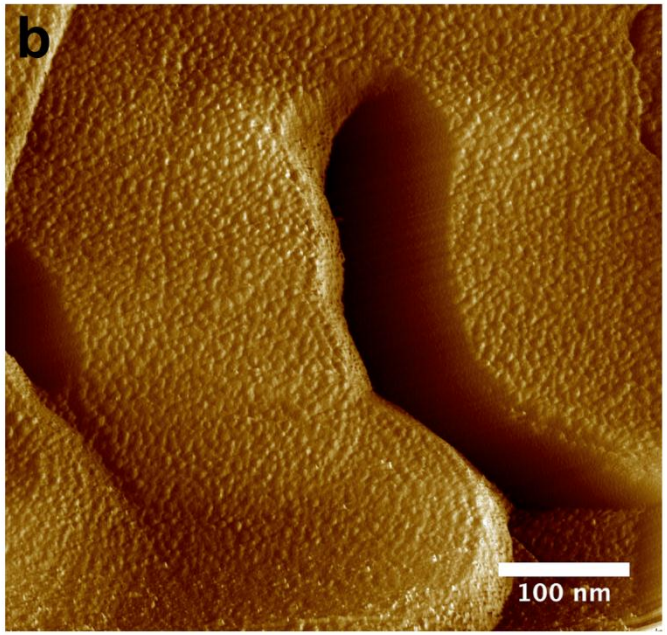
The first demonstration of its kind, providing evidence that these biotic components may be used for channeling electronic signals between synthetic devices and the electron transport chains of live cells, potentially leading to new biosystems that combine the replication, self-repair, and precise biochemical control of nature with the vast toolbox of synthetic materials and nanotechnology.

Scanning Tunneling Microscopy/Spectroscopy

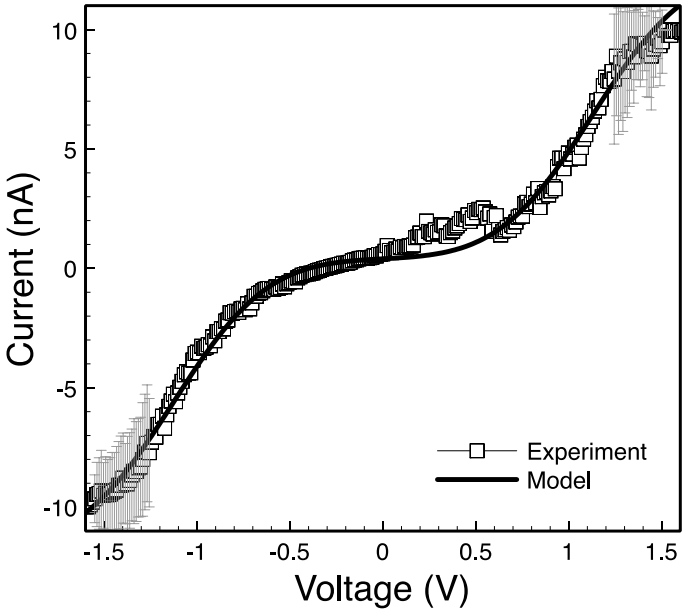
Individual multiheme cytochromes



Crystal structure of MtrF from Clarke *et al.* PNAS 2011



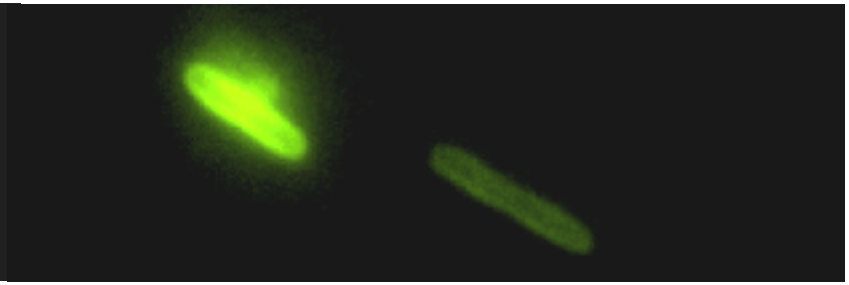
MtrF monolayer – the "bumps" are individual proteins



Agreement with the multistep hopping mechanism using known inter-heme spacings from the MtrF crystal structure

Next Phase (2013): Control of Electron Exchange via Bacterial Nanowires at Hybrid Living-Synthetic Interfaces
El-Naggar (USC)

Physiological switch: Developed methodology to physiologically induce bacterial nanowires in microfluidic devices as shown here by switching to extracellular respiration conditions.



Can we achieve a genetic switch?

Objective 1: Profile the underlying gene expression with time-dependent transcriptomic analyses (RNA-seq) during bacterial nanowire production. The knowledge gained may enable the future development of genetic switches for turning on/off/amplifying electron transfer.

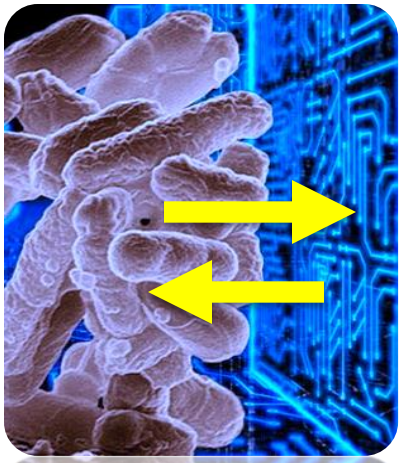
Can we "plug" cells directly to synthetic devices using this same methodology?

Objective 2: To direct and monitor the wiring of bacterial cells (*Shewanella oneidensis* MR-1) to micro/nano scale electrodes in microfluidic devices, while measuring the redox activity of the cells.

RNA-seq work in collaboration with Golbeck (Penn State)

Long-term goal: Synthetic biology approaches for transferring the extracellular electron transport function naturally existing in microbes like *Shewanella* to other cell types.

Next-generation sequencing

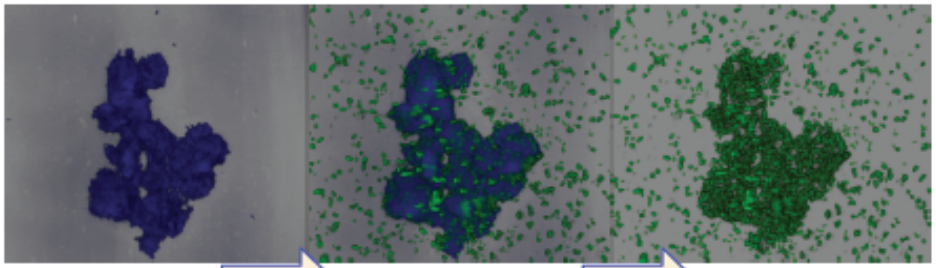


Long-term goal: Powering and interfacing to a synthetic device directly using cellular metabolic activity

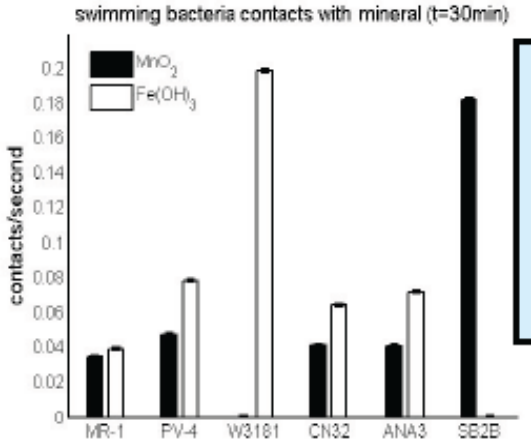
Bacteria Attach to Charged Surfaces Via Extracellular Electron Transport: Nealson

USC

(1) Bacterial sensing and response to charged surfaces was demonstrated using both insoluble minerals and charged electrodes



MnO_2 → Attachment → Biofilm Formation



(2) Different species of *Shewanella* interact preferentially with Fe or Mn oxides.

(3) Bacterial sensing and response is notably different for different bacteria at different potentials – and genes for extracellular electron transport (EET) are required.

	-200mV	0 mV	+200 mV	+400 mV	+700 mV
MR-1	-	-	-	30-60	50-80
W3-18-1	-	10-30	30-60	30-60	50-80
PV-4	-	-	10-30	30-60	30-60
ΔmtrB	-	-	-	-	-

(3) Response of 3 strains of *Shewanella* and one mutant defective in EET to electrodes with different charge potentials.

Outlook

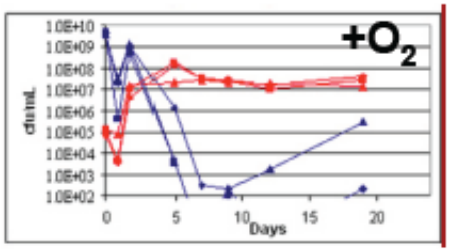
The first demonstration of bacteria finding and attaching to charged surfaces via a mechanism we call "congregation". It involves both genes for EET, and genes for sensing of the membrane potential. This mechanism is widespread, and important in microbial ecology in systems ranging from medicine to corrosion. Current work is aimed at mechanistic studies of congregation, and with using charged surfaces to study cellular interactions (bacteria/bacteria and bacteria/eukaryotes).

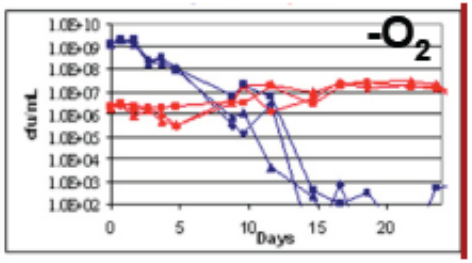
AFRL

Evolution & Survival: Effect of Different Electron Acceptor Environments: Finkel

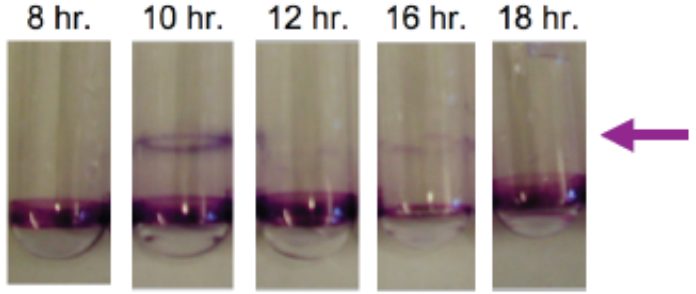
USC

(1) *Shewanella* evolve under both aerobic and anaerobic conditions as shown by appearance of more fit mutants (red) that outcompete unevolved parents (blue), though with different dynamics.

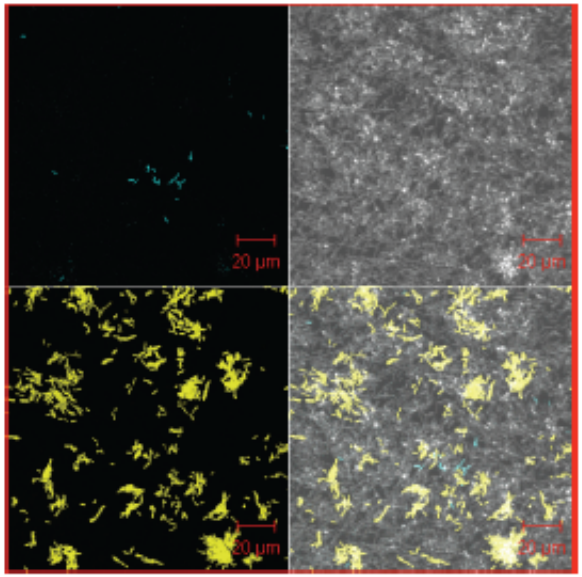




(2) Availability of terminal electron acceptors (oxygen and other compounds) affect biofilm formation over time. Upper biofilm (arrow) at air-medium interface; lower biofilm anaerobic.



(3) First demonstration of adaptive evolution in monospecies biofilms: aging biofilms of *E. coli* and *Shewanella* contain mutants with altered properties forming clumps of evolved cells (yellow); parental cells (blue).



This is the first demonstration of adaptive evolutionary change in *Shewanella*, also showing that the source of terminal electron acceptor affects the mode and tempo of evolution, in both the planktonic and biofilm lifestyles. The ability to form particular architectures and adapt within those structures has important implications for our understanding of extracellular electron flow in a variety of natural and artificial systems.

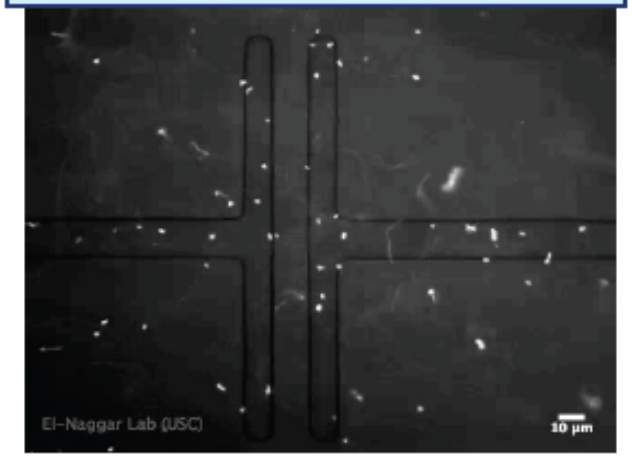
AFRL

Extracellular Electron Transport: Redox Interactions between Microbes & Surfaces

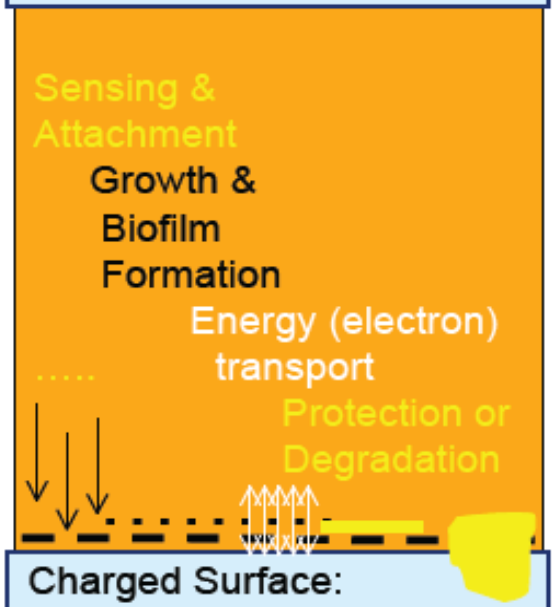
The Next Phase:

Microbial electron transfer in systems directly relevant to human performance: Host-Bacteria Interactions

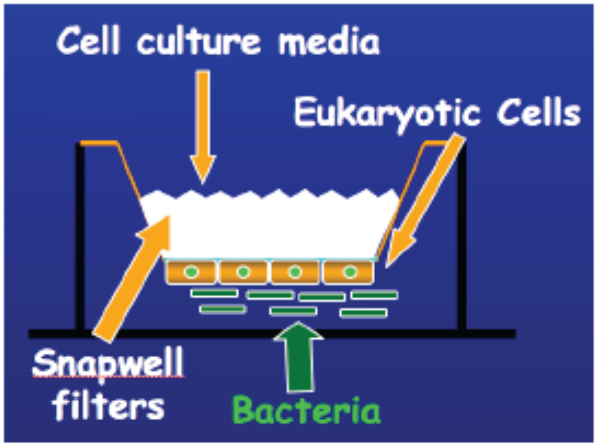
(1) **Microfluidic on-chip cultivation** with physical electrodes to control the microhabitats, redox conditions, and inter-species interactions, while investigating the interfacial electron transfer mechanisms employed by human-relevant microbes.



(2) Behavioral interactions of microbes with charged surfaces.



(3) Eukaryotic/Bacterial Tissue Co-Culture system allows aerobic incubation of mammalian cells above with culture of anaerobic bacterial cells below. Allows the control and study of terminal electron acceptor composition.

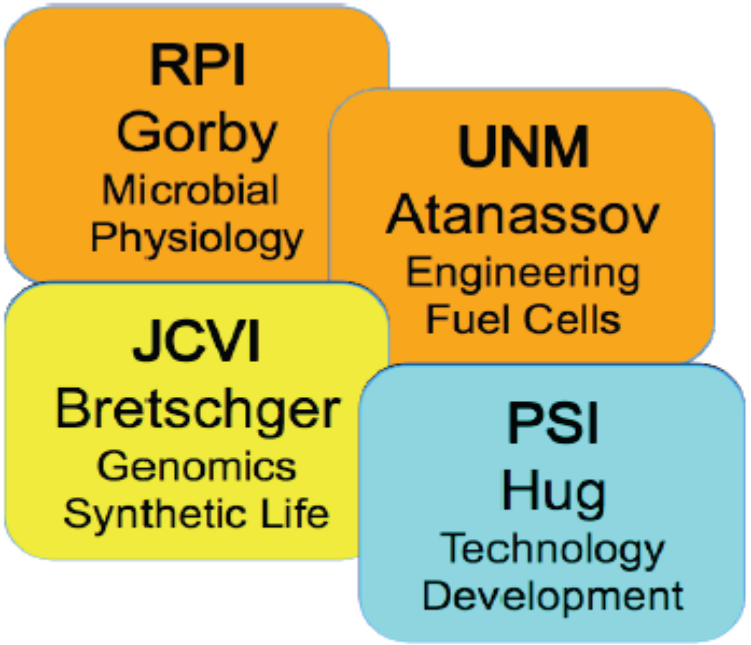


Bioengineered Fuel Cells: Optimization via Genetic Approaches & Multi-Scale Modeling

USC

USC Team:		GS	PD	Staff	UG	Pubs*
Nealson	Geology	5	3	1	4	11
Finkel	Biology	4			4	5
El-Naggar	Physics	4	2		4	10

Interactive Groups



Goals: **(1)** To understand the process of bacterial extracellular electron transport (EET) at multiple levels: physical mechanism; physiological controls; microbial behavior; and, adaptation and evolution.

(2) To expand this understanding from environmental microbes to those involved with human disease and/or performance.

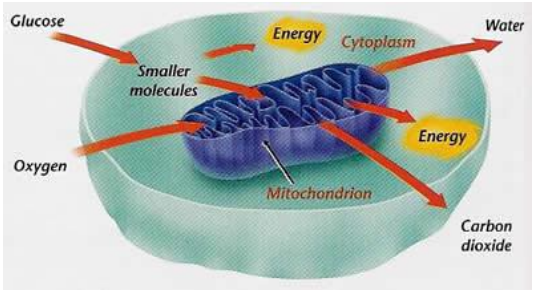
(3) To use modern approaches of genetics and synthetic life to exploit this knowledge.

*Co-authored papers: KN & ME-N, 5; KN &SF, 2

AFRL

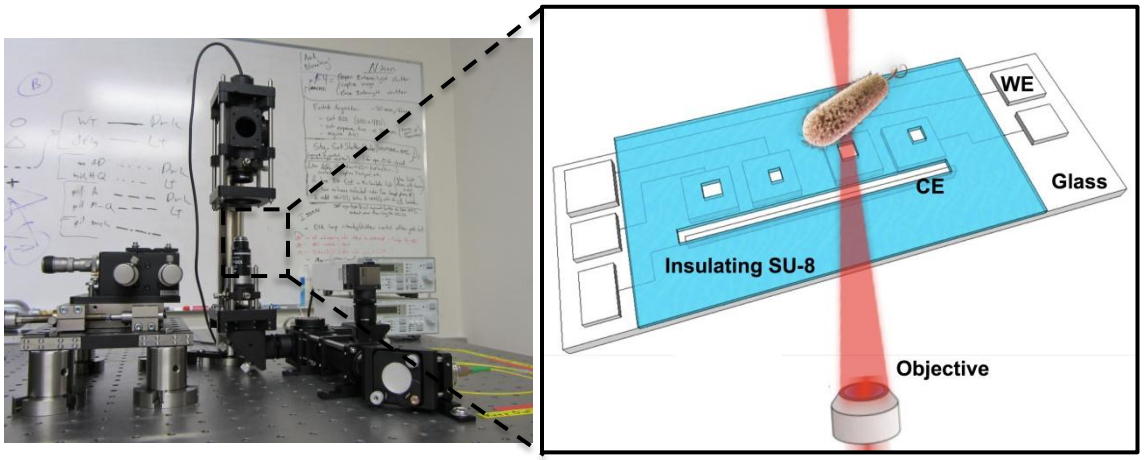
Photoelectric Stimulation of Mitochondrial Metabolism: Interfacing Individual Organelles to Electrodes

Mitochondria are the “powerhouses” responsible for electron transport and oxidative phosphorylation in the cell: A unique target for photo-electro-magnetic stimulation with potential for enhancing human performance e.g. hardening retinas of pilots to laser exposure (AFRL).

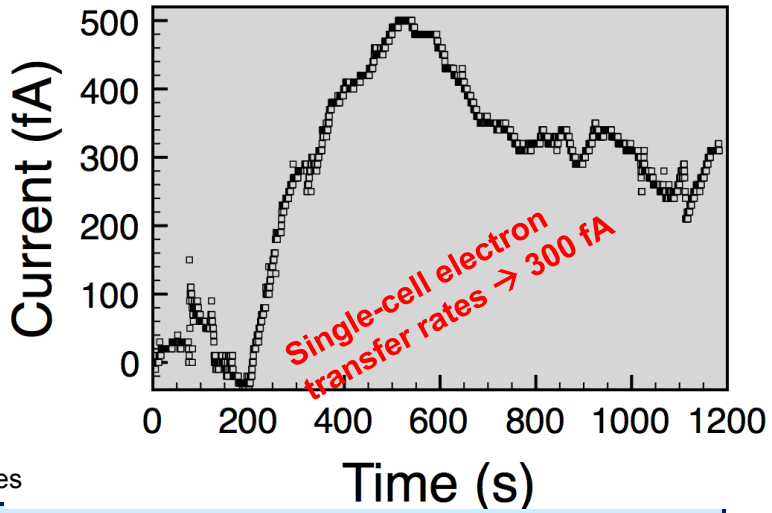


Objective: Discover the fundamental mechanisms linking photo-stimulation to metabolic (electron transfer) response of mitochondria, down to the level of a single mitochondrion (the limit of cellular metabolism).

Approach: Measure electron transfer from individual mitochondria (photo-stimulated and control) using a combined optical trapping and electrochemical platform previously developed for bacteria



Manipulation and ET measurements of individual microbes on microscale “landing pad” electrodes



Collaborative effort with complementary approaches: Macroscale mitochondrial electrodes (Minteer, Utah) and light absorption/mitochondrial function in retinal cells *in vitro* (Wigle, AFRL)

Synergistic Interaction of Neuroprotective and Neuropoietic Factors for Maximum Cognitive Capability under Sleep-Deprived Conditions

PI: Victor T. Chan, D.Phil. (711 HPW/RHDJ)

Objectives:

- Investigate feasibility of preventing adverse effects of sleep deprivation while simultaneously enhancing neuroplasticity.
- Exploit synergistic interaction of neuroprotective and neuropoietic factors for maximizing cognitive capability under sleep deprived conditions.
- Elucidate mechanism of synergistic interaction of neuroprotective and neuropoietic factors and develop effective and safe strategies for cognitive enhancement.

Technical Approach:

- Use of neuroprotective factors to prevent neuronal damage caused by sleep deprivation.
- Stimulation of neuropoiesis (neurogenesis and neuroplasticity) by neuropioetic / neurotrophic factors.
- Exploit synergistic interaction of neuroprotective and neuropioetic factors.
- Mechanism elucidation of synergy between neuroprotective and neuropioetic factors.

Accomplishments:

- 2013 New Start
- Animal protocol in preparation

DoD Benefit:

Effective and safe augmentation of warfighter cognitive capabilities under sleep deprived (or other stressful) conditions will ensure mission success.





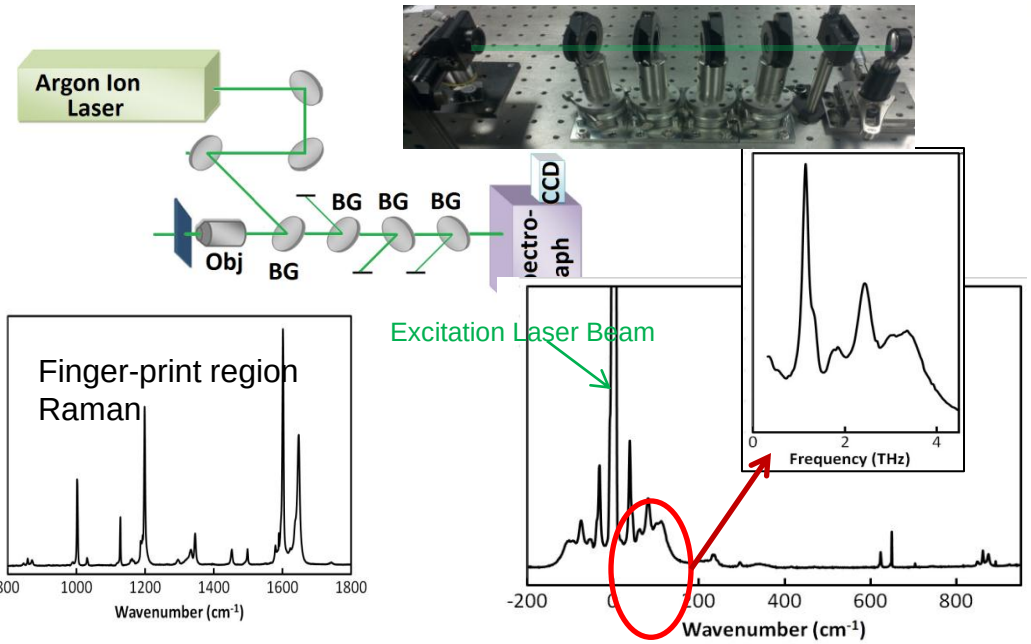


Probing Terahertz Resonance through Low-Frequency Raman: Hope Beier (AFRL/RHDO)

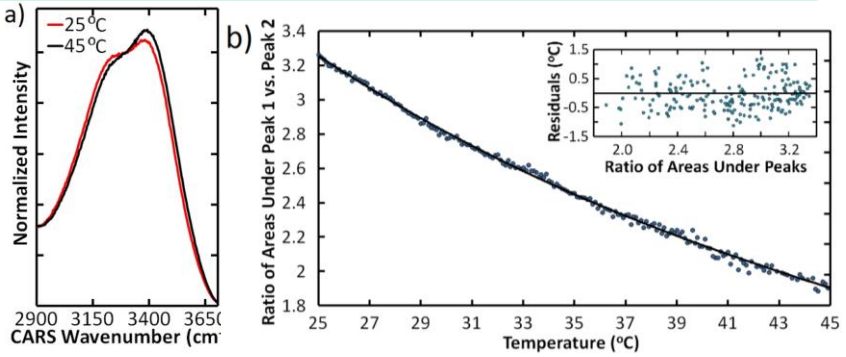
Objective: Use Raman scattering to obtain information about the susceptibilities of biomolecules, especially those in the THz region that cannot be obtained by probing with the frequencies directly.

Rationale: Biological response to resonance-type effects from THz may have unique characteristics that can be exploited to inhibit or activate cellular responses. Current knowledge of biomolecular absorption across THz frequencies is very limited. Traditional absorption techniques are limited in THz by low power sources and high background absorption by water. The use of visible light techniques escape these limitations and will identify the molecular fingerprint regions within the THz

Completed construction of a Bragg-grating-based low-frequency Raman system to detect THz



Coherent Raman used to map local temperature and monitor changes in molecular conformations



Future Work:

- Compare dry vs. aqueous samples
- Use of coherent Raman spectroscopy to monitor membrane disorder in cells and GUV
- Continue Raman vs. direct THz vs. thermal comparison

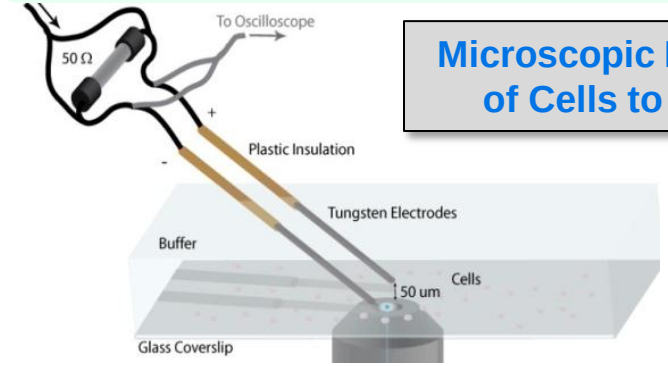
Activation of Intracellular Pathways by Nanosecond Pulsed Electric Fields:

Ibey (AFRL/RHDR)

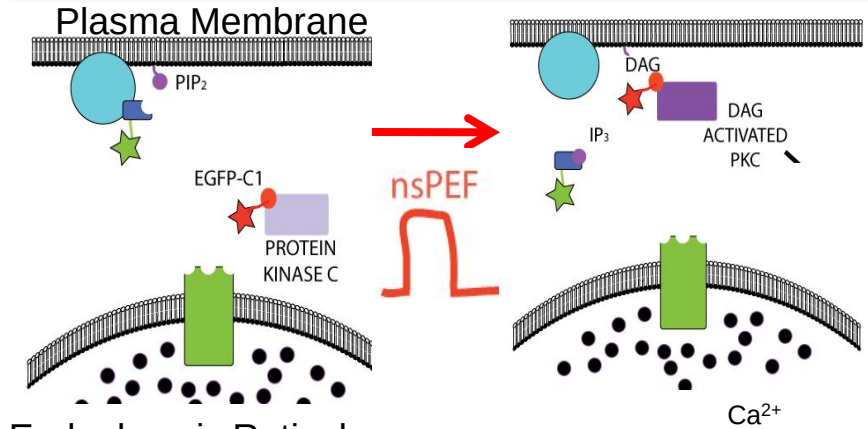
Overall Project Goal: To determine the underlying factors responsible for nsPEF sensitivity and downstream activation of intracellular pathways

Hypothesis: Nanosecond pulsed electric fields generate nanopores in the plasma membrane that activate intracellular pathways culminating in physiological stimulation

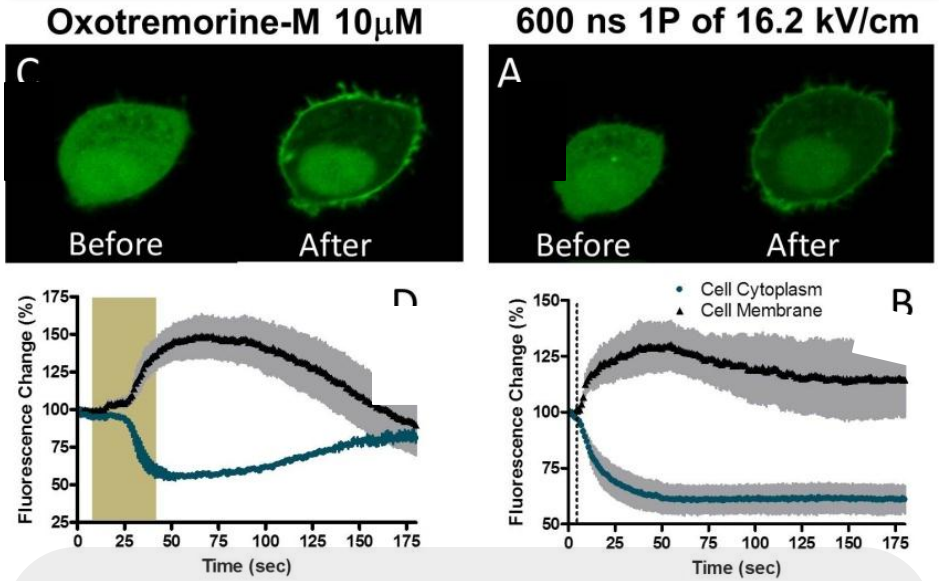
Microscopic Exposure of Cells to nsPEF



Fluorescent Tracking of PIP_2 Hydrolysis



PIP_2 Hydrolysis by Drug and nsPEF - *In Vitro*



Accomplishments:

- Obtained cell model to track PIP_2 hydrolysis by IP_3 release and DAG synthesis
- Generated a comprehensive data set illustrating the nsPEF-induced hydrolysis of PIP_2
- Generated data using receptor mediated drug

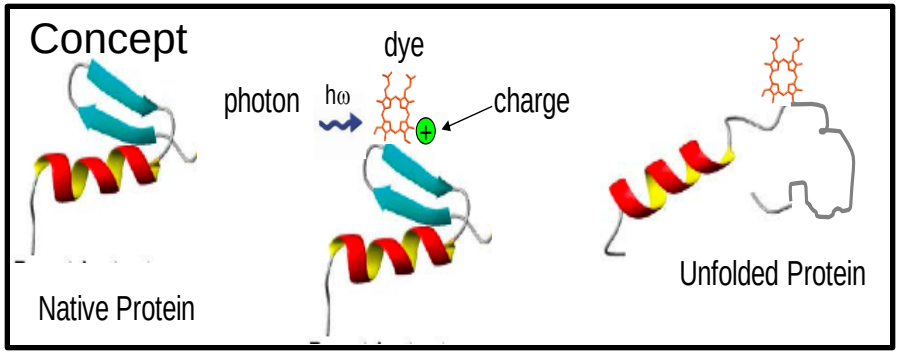
Future Experiments:

- Translate system in hippocampal neuron model
- Validate activation of protein kinase C

Mechanisms for photo-induced manipulation of protein functions

Thomas, Parker, McMicken, Rozinek (711 HPW/RHD)

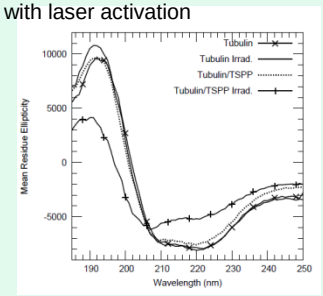
Objective: Demonstrate that photo-induced mechanisms (photo-induced electron transport (PET)) can trigger protein conformational changes that can be used to modify the structure and function of the polypeptides—manipulation of protein to serve as a nanoparticle with unique properties.



Working Hypothesis: Mechanism of light-activated manipulation of the protein structure facilitated by a porphyrin dye is predictable, reproducible, and is strongly localized within the structure

Evidence of PET-induced Conformational Changes

Circular Dichroism Shows change with laser activation

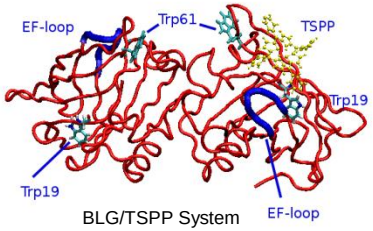


McMicken et al, In preparation (2013).

Potential Applications

- Non-Native sensory functions
- Non-Native recognition and catalytic functions
- Non-Native aggregative behavior or interaction with nanoparticles
- Knock-out of specific proteins to study cellular-pathways (link to other RHD missions/LRIRs)

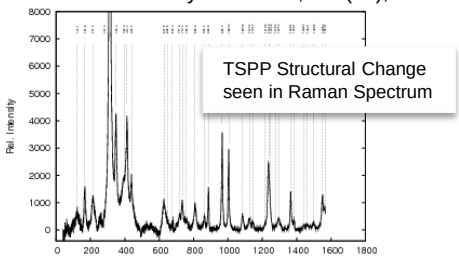
Methods



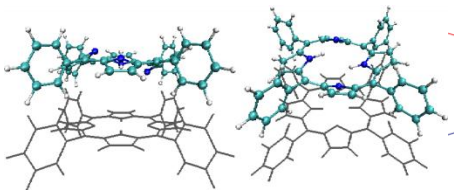
BLG/TSPP System

Predict binding site of porphyrin
– docking simulations
– symmetry breaking bending

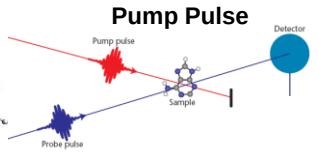
Parker et. al. J. Phys Chem B., 116(36), 2012

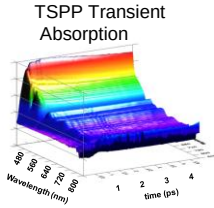


Evidence of bound configuration with theoretical simulation (Gaussian09) & Expt Raman spectroscopy/CD/abs

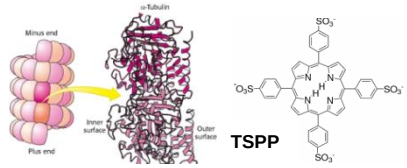


Augment numerical SERID (collaboration) light-interaction
– add charge transport mech.

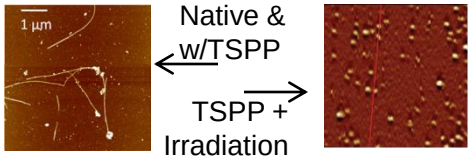




Observe kinetics of system in transient absorption spectroscopy experiment

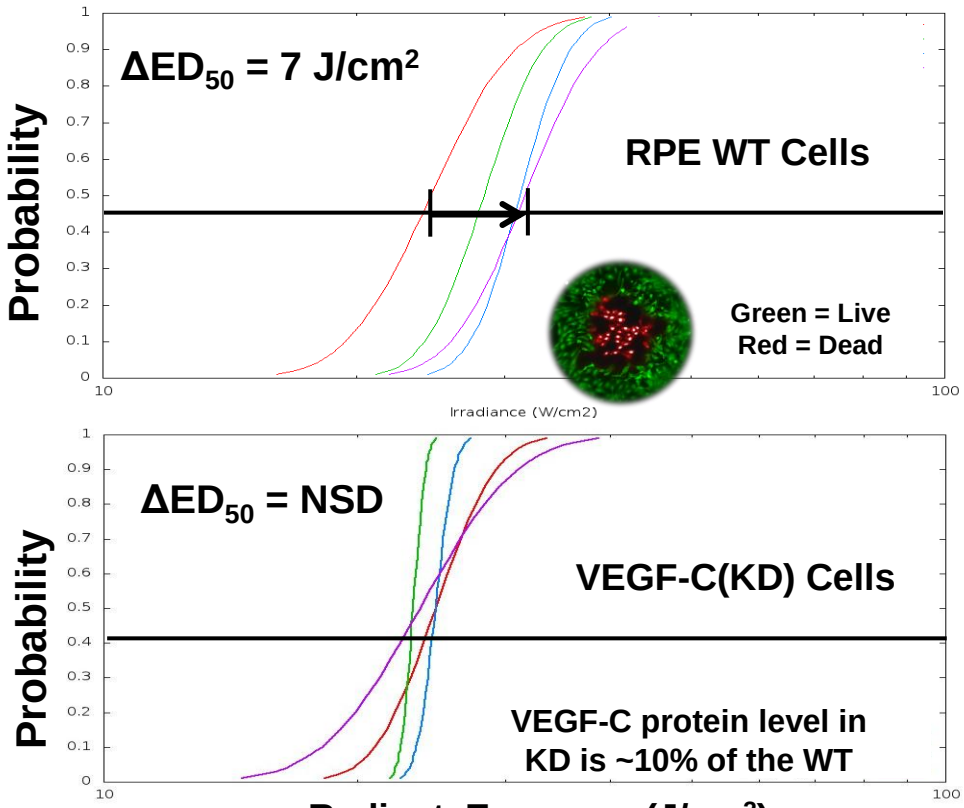


Candidate system for in-vitro Demonstration of cellular effect
– microtubule formation (tubulin)

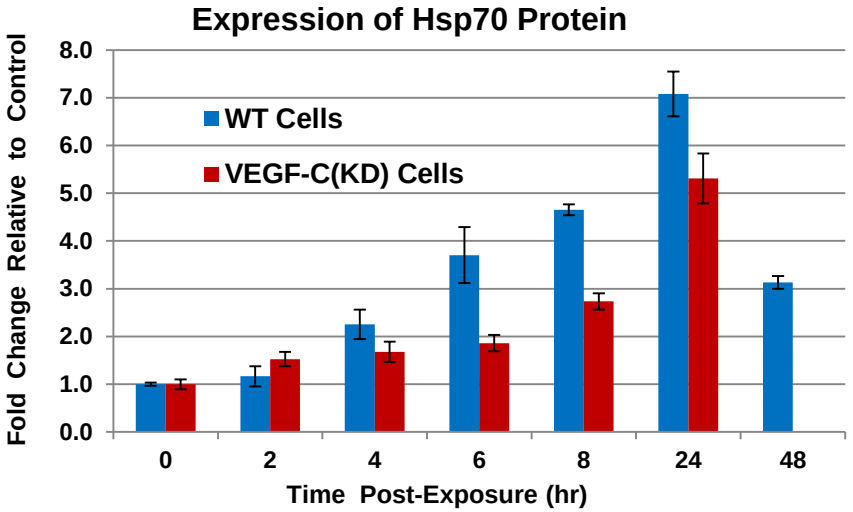


AFM Observation of polymer-ization disruption in microtubules

Mechanisms of Low Level Light Biostimulation for Enhancement of Performance and Protection



Line Color	Conditioning Exposure	Irradiance (mW/cm^2)	ED_{50} (J/cm^2)
Red	0	0.00	24.5
Blue	2.88 J/cm^2	0.40	31.3
Green	2.88 J/cm^2	0.80	28.4
Magenta	2.88 J/cm^2	1.60	31.7



	Growth Stimulation / Growth Control					Anti-Apoptosis		
	NF-κB	Cyclin D	ATP	Growth	VEGF-C	Bcl-2	Bcl-xL	Hsp 70
WT	↑	↑	↑	↑	↑	↑	↑	↑
VEGF-C(KD)	↔			↔	↔			↑
Mir-146a(-)	↑				↔			↑

	Pro-Apoptosis						Protection
	p53	Bax	FasL	miR-146a(-)	Casp 8	Casp 9	Adaptive Response
WT	↓	↓	↓		↔	↓	+
VEGF-C(KD)			↑	↔			−
miR-146a(-)			↑	↔			−

Future Direction

- Optimize response
  - Pulsed vs. continuous exposure / 810nm vs. 671nm
  - Genetic analysis of mutant strains
  - Modulate apoptosis
- Evaluate in animal model

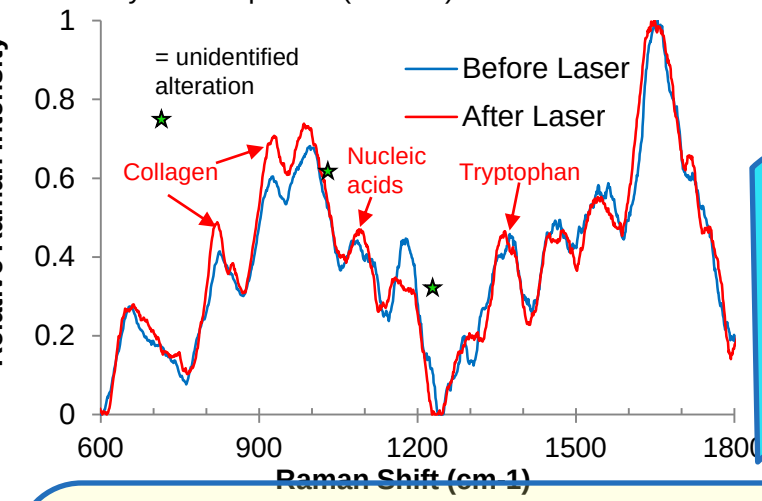
Physicochemical Consequences of Laser Exposure

Rockwell & Denton, AFRL/RHDO

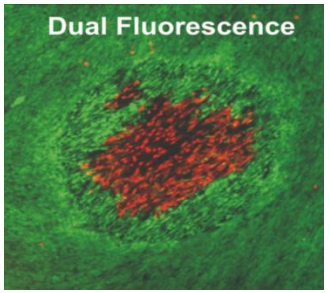
Goal: To enhance cell/organ resistance to thermal injury.

Raman spectral analysis (chemical groups) on the ***TRaF microscope (Thermal+Raman+Fluorescence)***

Identification of peaks (intracellular molecules) that are altered by laser exposure (thermal)



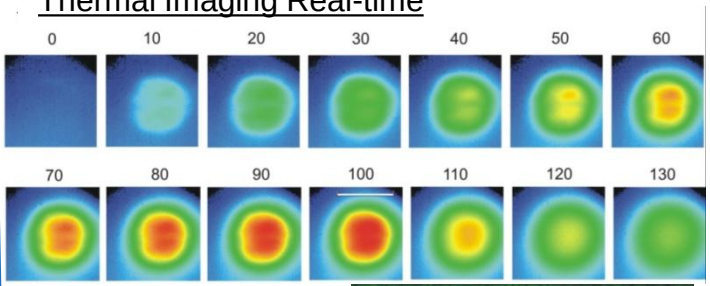
Damage Assessment

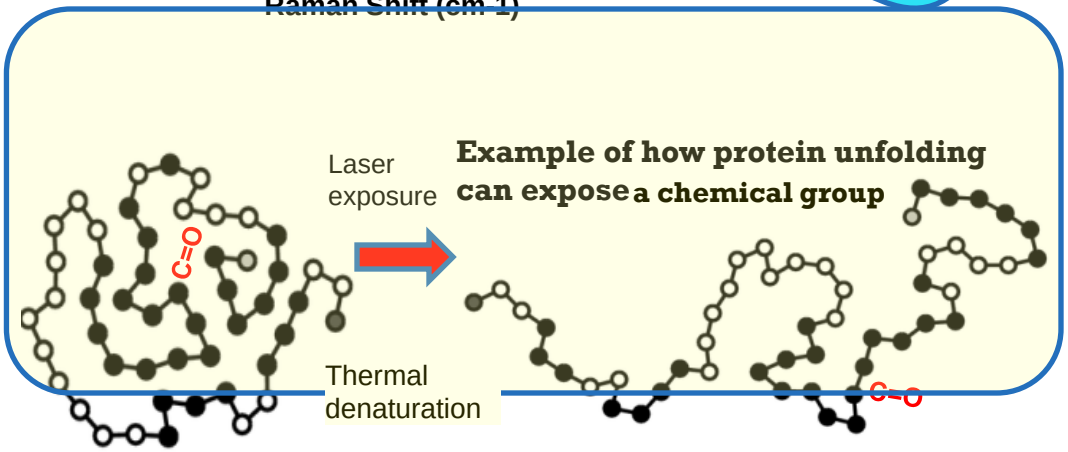


Real-Time TRaF Microscope
(ALL-IN-ONE IMAGING)

Thermal Mapping
Raman Mapping
Fluorescence (damage) Mapping

Thermal Imaging Real-time

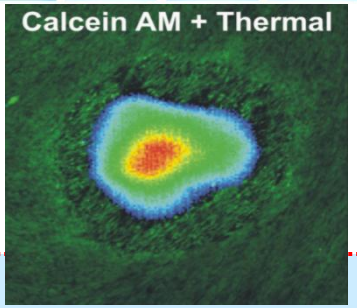




MICROTHERMOGRAPHY

Spatially resolved data showing thermally-dependent cellular damage.

Biomed. Opt. 16(3), 036003



Payoff to the Air Force:

1. *Physiologically-based resistance to laser injury*
2. *Reduction of requirement for laser eye protection*

Bioenergy: A Progressive Research Strategy

Generation	1st	2nd	3rd	4th
System Type	Natural Biosystems	Optimized Natural Biosystems	Hybrid Systems	Artificial Systems
Basic Research Type	Characterization Mechanisms Models	Metabolic/ Protein Engineering	Synthetic Biology	Chemistry & Materials Science
Disciplinary Inputs	Biology → Chemistry; Math ↔ Physics ↔ Engineering			

Comprehensive identification of genes required for algal photosynthesis and lipid accumulation

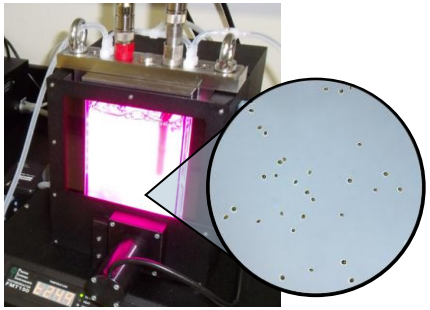
Martin Jonikas (Carnegie Institution for Science)

Goal: Identify gene targets for engineering to increase yields and lipid content

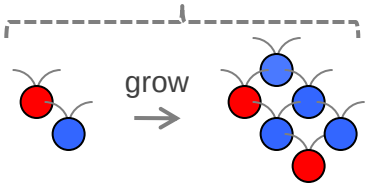
Methods: Our novel approach will allow characterization of **>100,000 mutants simultaneously**.

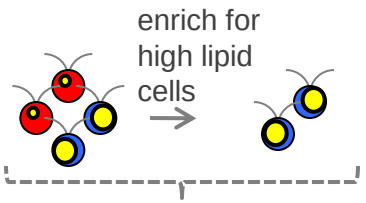
Results: Demonstrated for 1,000 mutants, preliminary data suggests >100,000 feasible

1,000 mutants growing in one culture

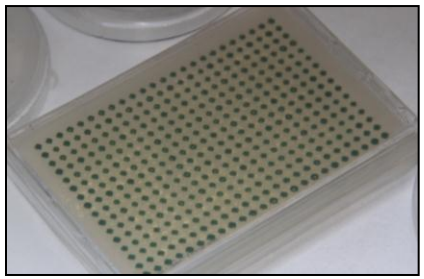


3. Measure growth rates



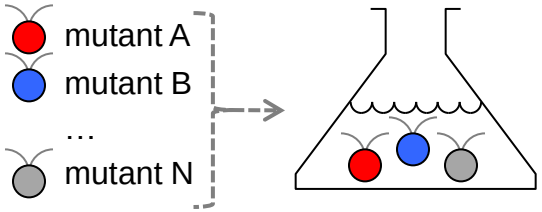


1. Generate thousands of mutants



Each colony on this plate is a mutant, each mutant has one broken gene.

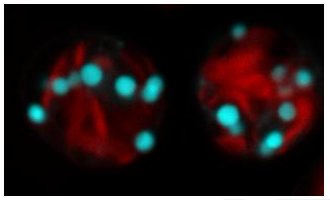
2. Combine mutants into one mixed culture



Each mutant carries a unique DNA “identity tag”. We can count tags to determine each mutant’s abundance.

4. Quantify lipid content

Microscopy of cells reveals lipid bodies



lipid

chlorophyll

Comprehensive identification of genes required for algal photosynthesis and lipid accumulation

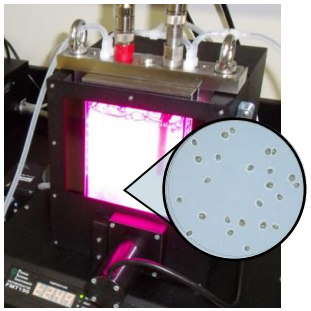
Martin Jonikas (Carnegie Institution for Science)

Goal: Identify gene targets for engineering to increase yields and lipid content.

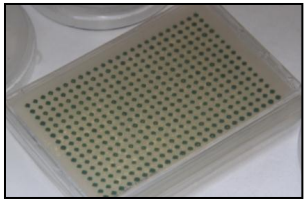
Methods: Novel tools allow characterization of tens of thousands of mutants simultaneously.

Results: Characterized >15,000 mutants. Isolated >50 mutants with perturbed lipid content and >50 mutants with defects in photosynthesis.

1,000 mutants growing in one culture



1. Generate thousands of mutants arrayed on plates

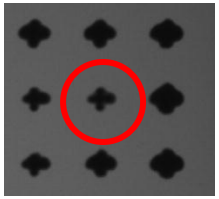


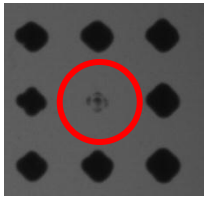
Each colony on this plate is a mutant, each mutant has one broken gene.

2. Measure growth rates on agar

food (acetate)

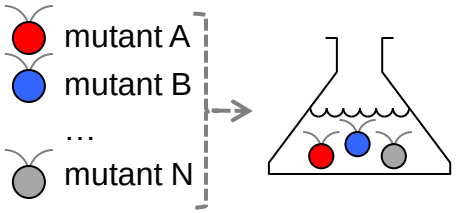
photosynthesis





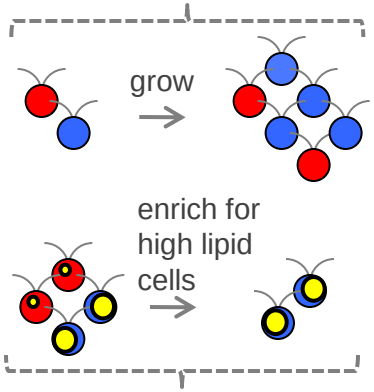
The circled mutant has a defect in photosynthetic growth

3. Combine mutants into one mixed culture

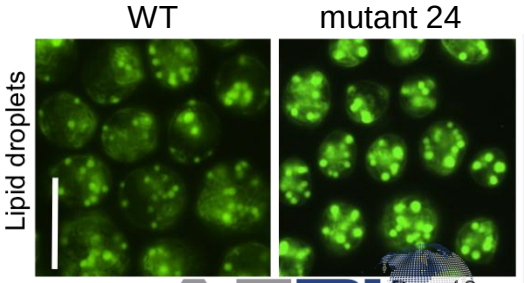


Each mutant carries a unique DNA "identity tag". We can count tags to determine each mutant's abundance.

4. Measure growth rates in liquid



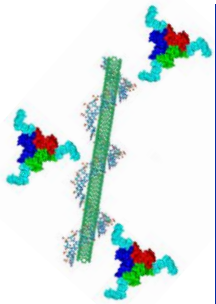
5. Quantify lipid content



Lipid droplet stain confirms the high lipid content of one mutant

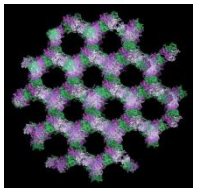
3-D Enzymatic Nanomaterial Architectures for Energy Harvesting
Columbia, U of Washington, U of New Mexico, U of Utah

Objectives:



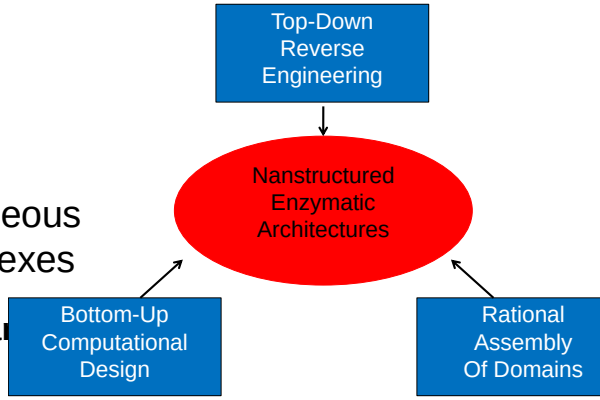
(1) Create advanced biomolecules, biocomplexes and bioassemblies that are tailor-made for self-assembly and ultimately optimized for device integration

(2) Develop top-down and bottom-up approaches to creating new nanomaterial architectures



(3) Design optimized systems for energy harvesting applications so that common pitfalls are addressed during development

Technical Approach:



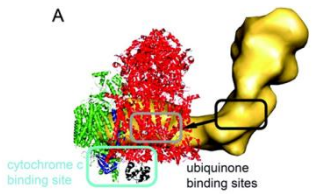
- Design heterogeneous biomolecular complexes
- **Design biomolecular interactions**
- Protein engineering for active site organization
- Create new nanostructred enzymatic architectures

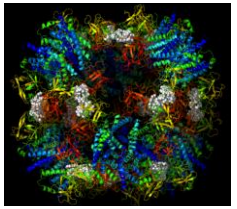
Accomplishments:

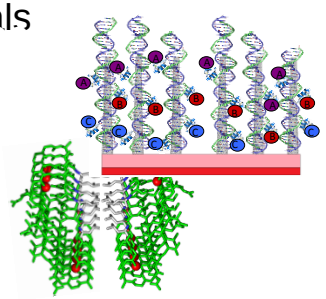
- Developed calcium-dependent cross-linking domains for enzymatic hydrogel formation
- Electrochemical evaluation of native electron transport chain metabolons at electrode surfaces
- Computationally designed *de novo* protein monomers, *de novo* repeat proteins, homo-oligomers, 2D protein layers, and a tetrahedral and octahedral cage

DoD Benefit:

Many advanced energy harvesting technologies will benefit from the optimization of the interface between biological components and nanoscale materials



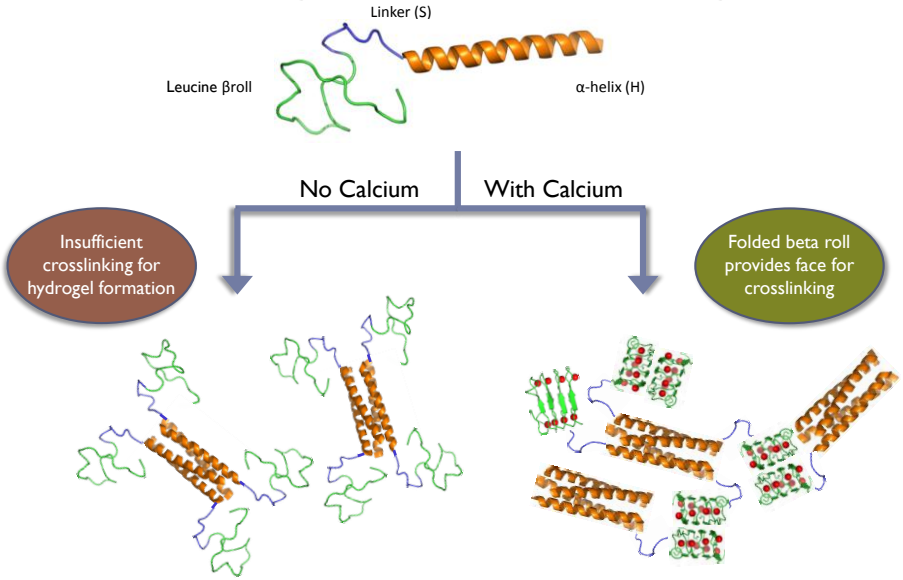




Engineering Protein and Nanomaterial Complexes and Assemblies

Scott Banta, Columbia University

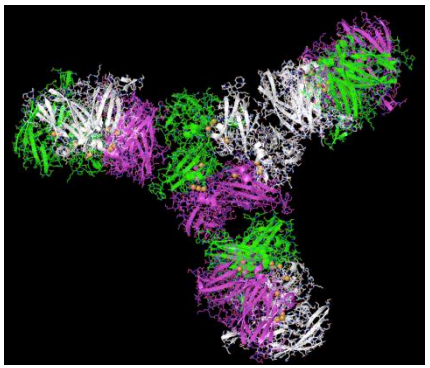
New Hydrogel Cross-Linking Strategy



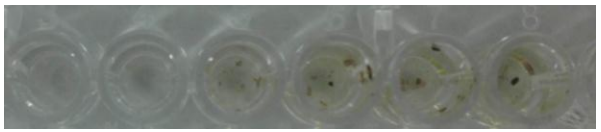
*A calcium-dependent peptide has been rationally engineered to serve as a cross-linking domain to produce stimulus-responsive protein hydrogels

*Laccase enzymes have been fused to carbon nanotube binding peptides

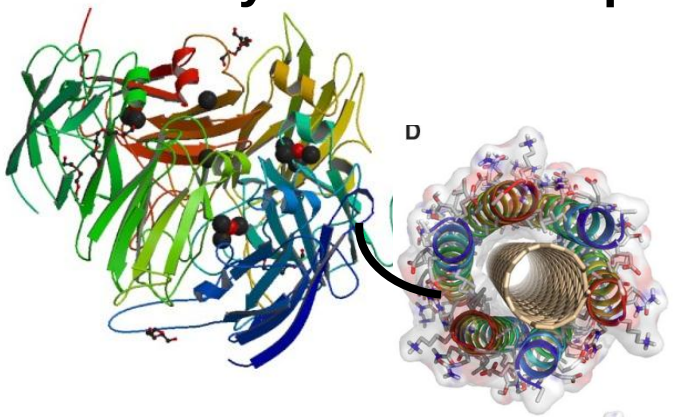
*A mutant laccase designed at UW self-assembles into active crystals



Designed Active Protein Crystals



New Enzyme/CNT Complexes



Enzyme-DNA-CNT Supramolecular Architectures for Functional Bio-Nano Assemblies

Plamen Atanassov, University of New Mexico - Spring Review FY13

Overview

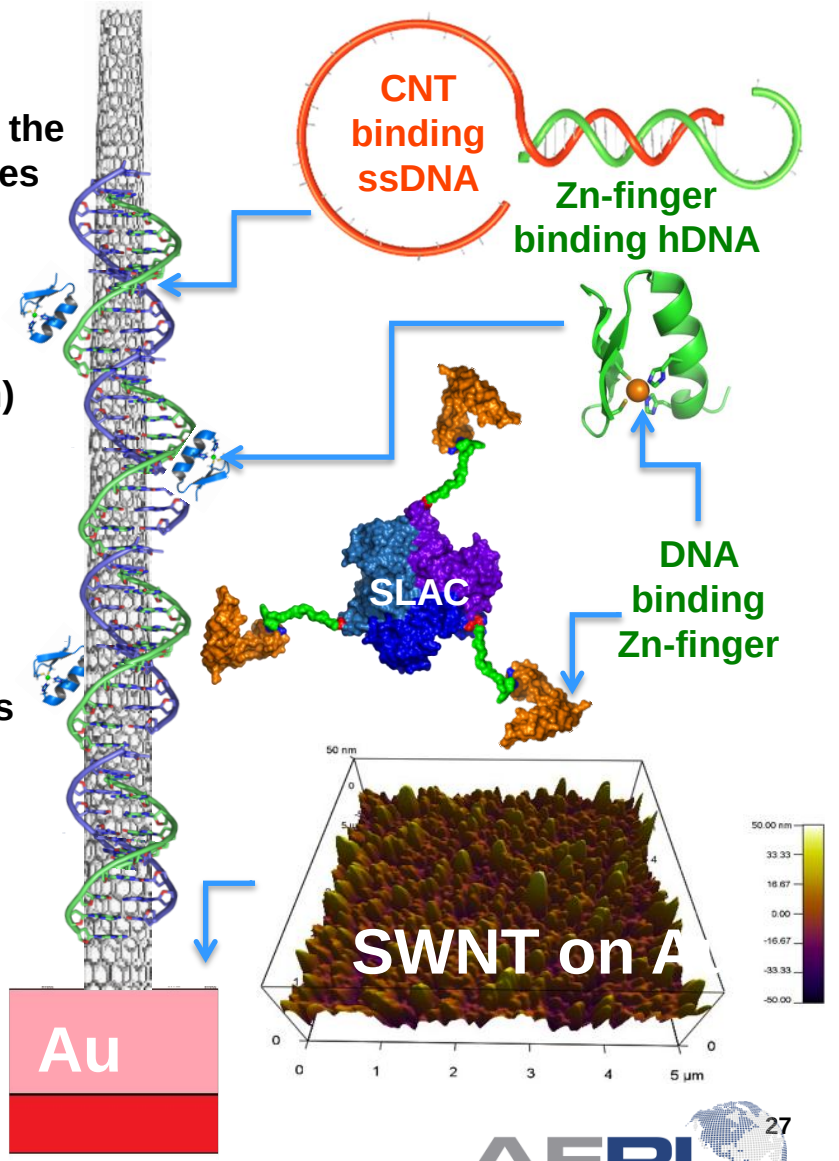
First year of the program was dedicated to establishing the tool-chest of the functional enzyme-DNA-CNT assemblies and preparing the groundwork for integration of the functional architectures.

Accomplishments

- ❑ Sourced and purified SW CNT or desired length (100 nm)
  - ✓ Photoluminescence of CNT suspensions
- ❑ Method of tethered attachment of CNT of Au surface
  - ✓ AFM characterization of CNT/Au architectures
- ❑ Design of the CNT and Protein binding DNA structure
  - ✓ Selected CNT binding ssDNA oligo-nucleotide
  - ✓ Selected Zn-finger binding hDNA fragment
  - ✓ Circular Dichroism Spectra of DNA-CNT constructs

In Progress

- ❑ Building CNT-modified Au surfaces of desired density
- ❑ Integrating DNA assemblies on the CNT-Au surface
- ❑ Tethering Small Laccase (SLAC) as a single enzyme model for the 3D nanostructured surface
- ❑ Identifying candidates for the first enzyme-channeling pair or triad to be tested in the surface architecture



Computationally Designed Biomolecular Interactions for Structured, Crystalline Self-Assembly

Baker Lab, University of Washington - Spring Review FY12

Overview

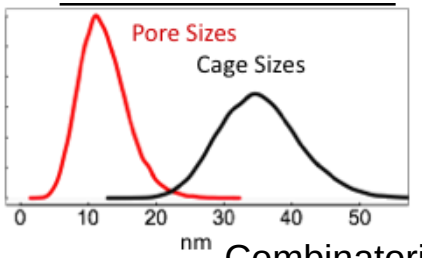
- Developing new tools: computational design of hierarchical, protein-based self-assembly
- Applying new tools: incorporation of functional components to yield novel and enhanced functionalities

Accomplishments

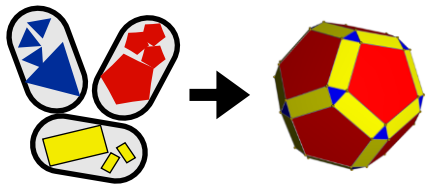
- Multi-component symmetry code added to Rosetta
- Extension of Rosetta Materials Design protocols:
  - Enhancements in docking:
    - New architectures: crystals, layers, cyclic oligomers, and two-component cages
    - Improved docking metrics
  - Modularized design and analysis code: rapid prototyping and access to new methods and metrics
  - Multi-component capabilities added to interface design and analysis code
- Successful designs to date: *de novo* protein monomers, *de novo* repeat proteins, homo-oligomers, a 2D protein layer, and a tetrahedral and octahedral cage

Toward Multi-Component Assemblies

Tailored Dimensions



Controlled Assembly



Combinatorial Functionalities

Structural	Structural	Structural
Catalytic	Catalytic	Catalytic
Recognition	Recognition	Recognition
Photoactive	Photoactive	Photoactive
Electronic	Electronic	Electronic

X X

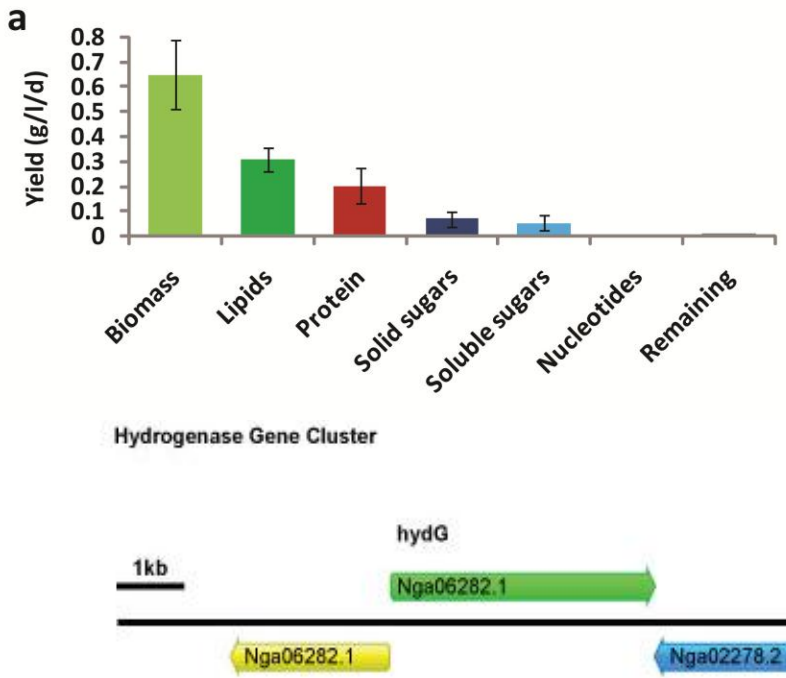
In Progress

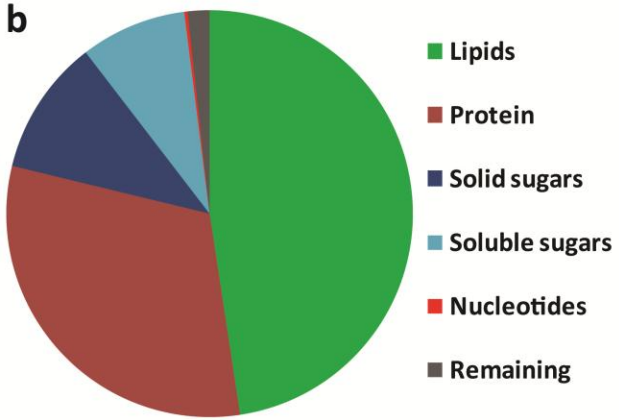
- Construction of a curated database of symmetrical protein building blocks
- Experimental characterization of designed two-component protein cages, cyclic-oligomers, layers, and disulfide-mediated crystals
- Extension of multi-component capabilities to crystal, layer, and fiber docking protocols

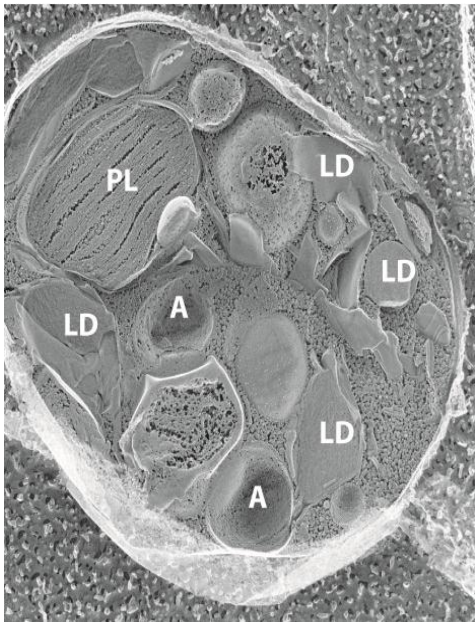
Developing an Improved Photosynthetic Refinery

(Posewitz Laboratory, Colorado School of Mines)

Objectives: Develop genetic tools for biotechnologically relevant energy transformation in marine algae and improve metabolic engineering strategies







Conclusions: Genome sequencing and characterization of *Nannochloropsis gaditana* demonstrates that this alga has among the highest photosynthetic conversion efficiencies of any marine alga characterized to date. Importantly, homologous recombination is feasible and photosynthetic flux can be directed to carbohydrates, hydrogen, lipids or protein. Radakovits et al., Nature Comm. 2012.

CSM Publications citing AFOSR funding during the last year

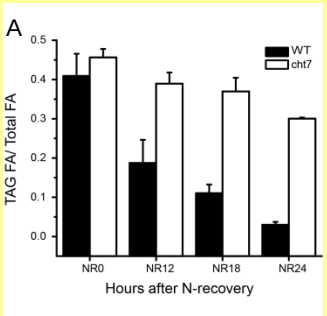
1. Meuser, J.E., D'Adamo, S., Jinkerson, R.E., Mus, R., Yang, W., Ghirardi, M.L., Seibert, M., Grossman, A.R., and Posewitz. M.C. (2012) Genetic disruption of both *Chlamydomonas reinhardtii* [FeFe]-hydrogenases: insight into the role of HYDA2 in H_2 production. *Biochemical and Biophysical Research Communications* 417, 704-709.
2. Catalanotti, C., Dubini, A., Subramanian, V., Yang, W., Magneschi, L., Mus F., Michael Seibert, M., Posewitz, M.C. and Grossman, A.R. (2012) Altered fermentative metabolisms in *Chlamydomonas reinhardtii* mutants lacking PFL1 and both PFL1 and ADH1. *Plant Cell* 24, 692-707.
3. Magneschi, L., Catalanotti, C., Subramanian, V., Dubini, A., Yang, W., Posewitz, M.C., Seibert, M., Perata, P., and Grossman A.R. (2012) A mutant in *ADH1* of *Chlamydomonas reinhardtii* elicits metabolic restructuring during anaerobiosis. *Plant Physiology* 158, 1293-1305.
4. Work, V.H., D'Adamo, S., Radakovits, R., Jinkerson, R.E., and Posewitz, M.C. (2012) Improving photosynthesis and metabolic networks for the competitive production of phototroph-derived biofuels. *Current Opinion in Biotechnology* 23, 290-297.
5. Peters, J.W., Boyd, E.S., D'Adamo, S., Mulder, D.W., Therien, J., and Posewitz, M.C. (2012) Hydrogenases, Nitrogenases, Anoxia, and H_2 Production in water-oxidizing phototrophs. In: Algae for Biofuels and Energy. Michael Borowitzka (Ed.), Springer, in press.
6. Mulder, D. W., Shepard, E.M., Meuser, J.E., Joshi, N., King, P.W., Posewitz, M.C., Broderick, J.B., and Peters, J.W. (2011) Structural insights into hydrogenase maturation. *Structure* 19, 1038-1052.
7. Cendron, L., Berto, P., D'Adamo, S., Vallese, F., Govoni, C., Posewitz, M.C., Giacometti, G.M., Costantini, P., Zanotti, G. (2011) Crystal structure of HydF scaffold protein provides insights into [FeFe]-hydrogenase maturation. *Journal of Biological Chemistry* 286, 43944-43950.
8. Radakovits, R., Jinkerson, R.E., Fuerstenberg, S.I., Tae, H., Settlage, R.E., Boore, J.L., and Posewitz, M.C. (2012) Draft genome sequence and genome transformation of the oleaginous alga *Nannochloropsis gaditana*. *Nature Communications*, 3:686. doi: 10.1038/ncomms1688.
9. Price, D.C., Chan, C.X., Yoon, H.S., Yang, E.C., Qiu, H., Weber, A.P.M., Schwacke, R., Gross, J., Blouin, N.A., Lane, C., Reyes-Prieto, A., Durnford, D.G., Neilson, J.A.D., Lang, B.F., Burger, G., Steiner, J.M., Löffelhardt, W., Meuser, J.E., Posewitz, M.C., Ball, S., Arias, M.C., Henrissat, B., Coutinho, P.M., Rensing, S.A., Symeonidi, A., Doddapaneni, H., Green, B.R., Rajah, V.D., Boore, J., and Bhattacharya, D. (2012) *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants. *Science* 335, 843-847.
10. Meuser, J. E., Boyd, E.B., Ananyev, G., Karns, D., Radakovits, R., Murthy N.U.M., Ghirardi, M.L., Dismukes, G.C., Peters, J. W., and Posewitz M.C. (2011) Presence and evolutionary significance of accessory FeS clusters in *Chlorella variabilis* NC64A [FeFe]-hydrogenase. *Planta* 234, 829-843.

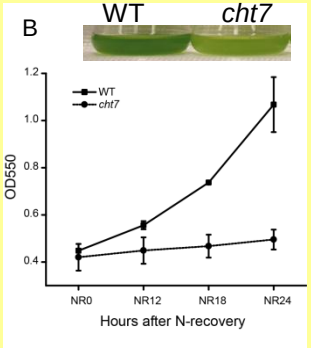
Regulation of Lipid Biosynthesis in Algae

C. Benning, Michigan State University

The microalga *Chlamydomonas reinhardtii* is used as a facile genetic model to study cellular energy metabolism. We aim to understand how cells regulate lipid droplet formation and degradation, which we experimentally control through nutrient supply.

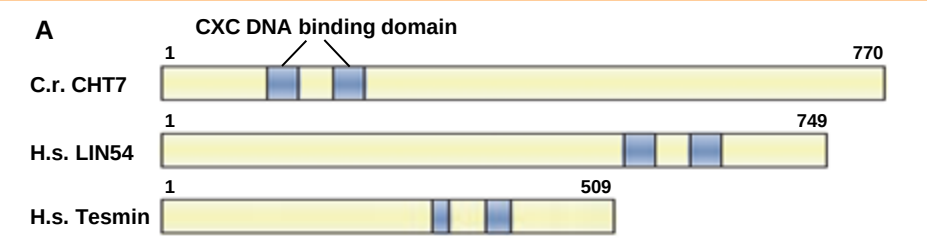
Triacylglycerols accumulate following nitrogen (N) removal and are degraded during N-resupply. This process is disrupted in the ***c****ompromised* ***h****ydrolysis of* ***T****AGs* ***7*** (*cht7*) mutant.



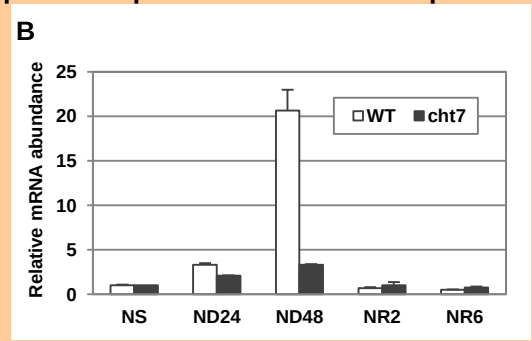


The mutant also does not resume growth following resupply with N, potentially linking lipolysis with cell proliferation.

CHT7 encodes a homologue of human LIN54, a component of an important regulatory complex with multiple cellular functions.

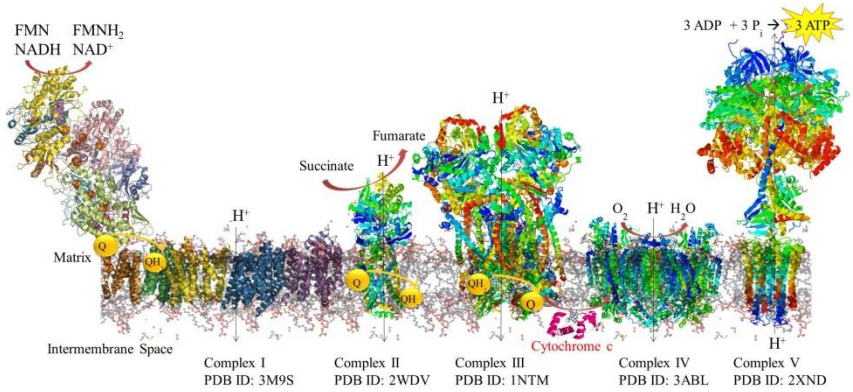


Global transcript analysis has revealed CHT7 targets including lipid droplet associated protein CrCGI-58.

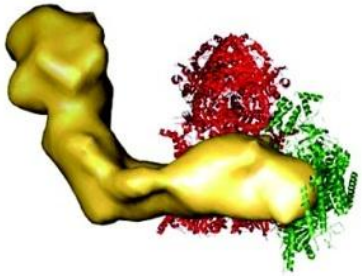


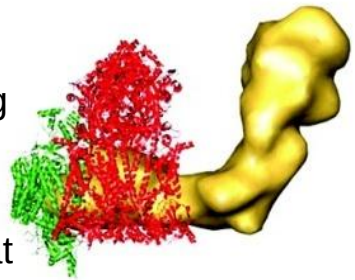
Understanding In-Situ and Ex-situ Formation of Metabolons

Shelley D. Minteer, University of Utah



In-Vivo Formation of Electron Transport Chain Metabolon

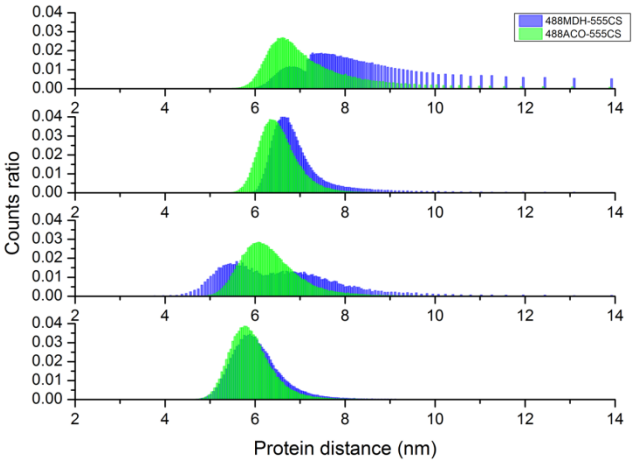


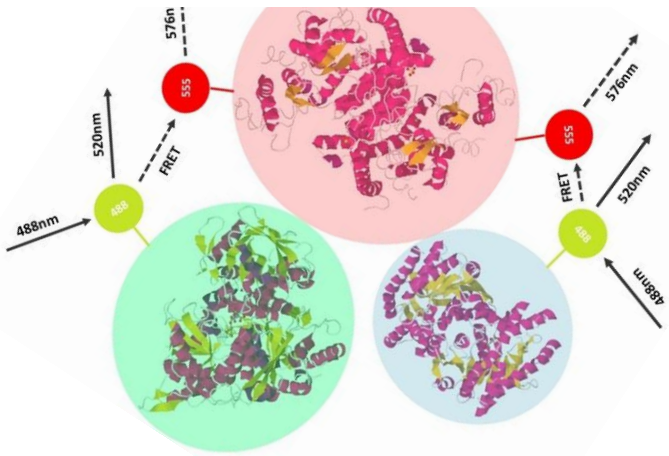


*Development of FRET microscopy techniques for studying the formation of metabolons and quantifying enzyme-enzyme-enzyme distances

**Ex-situ* bilayer techniques for forming electron transport chain metabolons

*Electrochemical evaluation of electron transport chain metabolons at electrodes





Quantitative FRET Microscopy to Differentiate Normal Complexation from Metabolon Formation